Measurement of Plasma Nortriptyline Concentrations: Radioimmunoassay and Gas-Chromatography Compared

J. D. Robinson,1 R. A. Bralthwaite,2 and S. Dawling2

We compared measurement of plasma nortriptyline by a recently developed radioimmunoassay technique with values obtained by traditional gas chromatography. The coefficient of correlation (r) was 0.98 for 84 samples from 21 controlled patients; for a separate series of 45 samples from 34 patients who were receiving in addition other medication, r was 0.96. Use of radioimmunoassay in the routine determination of plasma nortriptyline concentration is discussed.

Individual patients, even though they may be receiving the same dose of drug, demonstrate large differences in steady-state concentrations of tricyclic antidepressants in plasma (1–3). Because of the relationship between plasma concentrations and clinical effects, information on antidepressant concentrations achieved by individual patients is of considerable importance (2, 4–9). For nortriptyline, a therapeutic range of 50–150 μg/liter has been recommended to maximize antidepressant action (5, 9); higher concentration are associated with an increased incidence of adverse effects (10, 11). The routine determination of nortriptyline in plasma has therefore become important in monitoring treatment with antidepressant medication. Numerous methods for measuring tricyclic antidepressants in plasma have been described, many involving solvent extraction followed by gas chromatography. Radioimmunoassay has gained widespread use in the routine measurement of drugs such as digoxin and phenytion. A radioimmunoassay for tricyclic antidepressants in plasma recently described by Aherne et al. (12) has been applied to the determination of amitriptyline (13) and nortriptyline (14–16). Here, we compare this radioimmunoassay of plasma nortriptyline with a traditional gas-chromatographic method and report its application to the routine measurement of plasma antidepressant concentrations in patients undergoing treatment.

Patients and Methods

Samples. Patients’ plasma samples were obtained from two groups of psychiatric patients who were receiving antidepressant treatment with nortriptyline. The first consisted of a closely controlled group of inpatients who were given both single and multiple doses of nortriptyline. The only additional medication received by this group was diazepam and nitrézepam. The second consisted of both inpatients and outpa-

tients who were receiving routine antidepressant treatment with nortriptyline, but whose additional medication was not strictly controlled. Blood samples from all patients were collected into tubes containing lithium heparin as anticoagulant, the plasma being separated by centrifugation and stored at −40 °C before analysis.

Gas-chromatographic determination of nortriptyline. The method we used was a modification of that described by Kragh-Sørensen et al. (17). The procedure involved extraction of drug and internal standard (maprotiline) into 6 ml of n-hexane from 1-ml plasma samples made alkaline by the addition of 1 ml of pH 10 borate buffer. After back extraction into 1 ml of 50 mmol/liter sulfuric acid, drug and internal standard were re-extracted into 2 ml of hexane after addition of 100 μl of 4 mol/liter sodium hydroxide. We derivatized nortriptyline and maprotiline by reaction with 100 μl of heptafluorobutyrlic anhydride (HFBA) for 20 min at 70 °C. Excess reagent and solvent were removed by evaporation under a stream of air at 60 °C. The residue was reconstituted in 200 μl of hexane, and 1–3 μl aliquots were injected onto the column of a Hewlett Packard Model 5710 A gas chromatograph fitted with a linear electron capture detector. The column (1.8 × 4 mm i.d.) was packed with 3% SP2250 (Supelco Inc.) and run at 250 °C with the detector at 300 °C and a carrier gas (argon/methane; 95/5) flow rate of 60 ml/min. Under these conditions the retention times of nortriptyline-HFB and maprotiline-HFB were 3.2 and 4.8 min, respectively. The ratio of the peak heights of nortriptyline-HFB and maprotiline-HFB in plasma samples was compared with that of standards prepared from bovine plasma. Each assay was performed in duplicate. The reproducibility of the method was assessed by repeated analysis of a pooled sample taken from patients receiving nortriptyline medication. The mean value for 22 replicate analyses was 72 μg/liter with a coefficient of variation of 1.8%.

Radioimmunoassay of nortriptyline. We performed the assay using established radioimmunoassay procedures (18) according to the protocol outlined in Table 1. Tritiated amitriptyline (specific activity = 10.0 kCi/mol; 159.5 nmol/liter) was used as the label, and all dilutions of the reagents were made with the assay buffer, 0.1 mol/liter phosphate-buffered isotonic saline, pH 7.4. The antiserum, raised in a sheep against a nortriptyline–bovine serum albumin immunogen (12), was used at a final dilution of 1400-fold.

Nortriptyline hydrochloride standards were prepared from an ethanolic stock solution (80 mg/liter) serially diluted in the assay buffer to give concentrations ranging from 400 to 0.8 μg/liter. Plasma samples for the assay were diluted fivefold in buffer before analysis. Both standards and samples were analyzed in duplicate, with blank determinations for each sample.

The reagents were added to the assay tubes in the order
Table 1. Protocol for the Radioimmunoassay

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Total counts tube</th>
<th>Non-specific binding tube</th>
<th>Std. tube</th>
<th>Sample tube</th>
<th>Sample blank tube</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>200</td>
<td>200</td>
<td>—</td>
<td>100</td>
<td>200</td>
</tr>
<tr>
<td>[3H]Amitriptyline</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Standard</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Sample</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Normal plasma</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>—</td>
</tr>
<tr>
<td>Antiserum</td>
<td>—</td>
<td>—</td>
<td>100</td>
<td>100</td>
<td>—</td>
</tr>
</tbody>
</table>

Incubate for 1 h at room temperature and for 15 min at 4 °C

Vol μl

| Buffer | 200 | —   | —   | 200 | 200 |
| DCC a  | —   | 200 | 200 | 200 | 200 |

*DCC = dextran-coated charcoal; 12.5 g of Norit. A charcoal (Sigma) and 1.25 g of Dextran T-70 (Sigma) per liter of buffer.*

indicated in the protocol. After addition of the antiserum, the contents of the tubes were mixed and incubated at room temperature for 1 h. The tubes were then placed in ice-water for 15 min and cold dextran-coated charcoal was added for phase separation. The contents of the tubes were then vortex-mixed and the tubes centrifuged at 2500 rpm for 5 min at 4 °C; aliquots of the supernates were then taken for liquid scintillation counting.

Concentrations of nortriptyline in the unknown samples were interpolated from the standard curve (Figure 1) (19). A quality-control sample containing 90.0 μg/liter, analyzed in each batch of assays, gave a mean value of 87.8 μg/liter, with a CV, over five assays, of 9.8%. Within-assay variance for the radioimmunoassay was 4.7%. The avidity constant of the antiserum for nortriptyline was 9.3 × 10^7 liter mol⁻¹ and the assay could detect concentrations as low as 0.86 μg/liter in a 100-μl aliquot. Cross-reactivity studies indicated that the metabolites of nortriptyline did not interfere in the assay, and that only amitriptyline, imipramine, and protriptyline showed any degree of cross reaction (130, 80, and 61%, respectively); cross reaction with desipramine was less than 5%.

Results

Figure 2 shows correlation between plasma nortriptyline values obtained by the two methods for a group of 84 samples obtained from 21 “controlled” inpatients. As can be seen, there was an excellent correlation between the two methods (r = 0.98) for this set of samples. Figure 3 shows the correlation obtained for a separate series of 45 samples obtained from an uncontrolled group of 34 patients. The correlation obtained in this group of patients (r = 0.96) was almost as good as that obtained for the previous set of samples. This latter group of uncontrolled patients were also receiving additional medication (Table 2). The agreement between the two methods in both sets of samples (Figures 2 and 3) was good at both low (<50 μg/liter) and high (>150 μg/liter) plasma nortriptyline concentrations. In only six of the 129 plasma samples measured was there some discrepancy between values obtained by the two methods. Of these, only three might have produced a different clinical decision; all of the three were obtained in the uncontrolled group of patients, for whom cross reaction could account for a different value being obtained by radioimmunoassay. However, in only one of these results was the value obtained by radioimmunoassay higher than that obtained by chromatography.

Discussion

Routine measurement of tricyclic antidepressant drugs such as nortriptyline has become increasingly important. Gas chromatography, the usual technique, generally requires plasma samples in excess of 2 ml and is laborious and time-consuming.
consuming. As an example, the chromatographic method we used here involved a lengthy solvent extraction and back-extraction procedure, followed by derivative formation, and, finally, the chromatographic separation and electron-capture detection. Thus the number of samples that can be routinely processed by this procedure during a working day is severely limited. On the other hand, in the case of the immunoassay procedure described, no sample preparation other than a simple dilution is required, and upwards of 40 samples can be done in duplicate with appropriate standardization during a 4–5 h working period, excluding counting time. Automated liquid-scintillation equipment with computer-programmed data handling speeded the radioimmunoassay procedure and plasma nortriptyline values were directly calculated. The total sample volume required for the immunoassay procedure is only 100 μl.

The unequal cross reactivity of the antiserum for nortriptyline and amitriptyline suggest that the assay would not be ideal for the accurate determination of total tricyclic concentration in patients being treated with amitriptyline. The assay could, however, be used to assess patient compliance and to give an indication of the plasma drug concentrations.

The close correlation obtained between the two methods for nortriptyline, even in patients receiving other drug treatment, justifies the routine use of a nortriptyline radioimmunoassay. The sensitivity of the radioimmunoassay procedure, coupled with the possibility of assaying many samples without time-consuming work-up procedures further recommends the technique for phamacoconomic studies.

In the case of nortriptyline, most studies have shown that its concentrations in plasma are related to therapeutic as well as toxic effects (4, 5, 8, 9–11). A therapeutic "window" of 50–100 μg/liter is therefore recommended, to maximize antidepressant effects (5, 9). Because of the very large interindividual differences in plasma nortriptyline concentrations that may be obtained in the case of patients receiving routine medication, such measurements are essential to improve treatment. With the simple radioimmunoassay procedure described here, plasma nortriptyline concentrations could be measured more easily and individual dosage regimes optimized.

We thank Drs. S. Montgomery and J. L. Crammer for providing the blood samples for this study, Dr. G. W. Aherne and Guildhay Antisera for providing the antiserum, and Dr. A. Jørgensen (Lundbeck Ltd.) for providing the [3H]amitriptyline.

References